

## THE ANTI-INFLAMMATORY ACTIVITY OF THE LEAVES OF *COLOCASIA ESCULENTA*

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لقد تمت دراسة التأثير المضاد للالتهابات للخلاصة الإيثانولية لأوراق نبات كولوكاشيا إيسكيولينتيا في فئران ويستار باستخدام الاستسقاء المستحث بطحلب كاراجين في اليد اليسرى للألية وذات الجنب المستحثة بطحلب كاراجين والورم الحبيبي المستحث بكربة القطن. لقد تمكنت الخلاصة الإيثانولية من كبح الاستسقاء المستحث بطحلب كاراجين في يد الفأر. كما تبين أن هناك تأثير كايح على ارتحال الخلية البيضاء وخفض في إفرازات ذات الجنب وكذلك انخفاض في وزن الورم الحبيبي في طريقة الورم الحبيبي لكريات القطن. هذه النتائج تدل على أن الخلاصة الإيثانولية قد أنتجت نشاطاً مضاداً للالتهابات وذلك عند مقارنتها مع العياري والضابط غير المعالج.

Anti-inflammatory activity of the ethanolic extract of the leaves of *Colocasia esculenta* Linn. was studied in wistar rats using the carrageenan induced left hind paw edema, carrageenan induced pleurisy and cotton pellet induced granuloma model. The ethanolic extract (100 mg/kg, p.o.) inhibited carrageenan induced rat paw edema. It also showed an inhibitory effect on leukocyte migration and a reduction on the pleural exudates as well as reduction on the granuloma weight in the cotton pellet granuloma method. The results indicated that the ethanolic extract produced significant ( $p < 0.05$ ) anti-inflammatory activity when compared with the standard and untreated control.

**Keywords:** Anti-inflammatory, *Colocasia esculenta*, cotton pellet

### Introduction

*Colocasia esculenta*, commonly known as Taro, belongs to the family Araceae. The leaf juice of the plant is styptic, stimulant and rubefacient, and is useful in internal haemorrhages, otalgia, adenitis and buboes. The juice of the corm is laxative, demulcent and anodyne (1). Prolonged uses of both steroidal and non-steroidal anti-inflammatory drugs are well known to be associated with peptic ulcer formation (2). Hence, search for new anti-inflammatory agents that retain therapeutic efficacy and yet are devoid of these adverse effects is justified. There is much hope of finding active anti-rheumatic compounds from

indigenous plants as these are still used in therapeutics. Herbal drugs are being proved as effective as synthetic drugs with lesser side effects.

The enzyme, phospholipase A2, is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes which by attracting polymorphonuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase A2 converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cyclooxygenase (prostaglandin synthase) to prostaglandins, which are major components that induce pain and inflammation (3, 4). Arachidonic acid is also converted to leukotrienes via lipoxygenase enzyme. The present study is therefore an attempt to assess the efficacy of this indigenous herb for its anti-inflammatory activity in rats.

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## Materials and Methods

### *Plant Material:*

The leaves of *Colocasia esculenta* were collected from the local areas of Belgaum, identified and authenticated by Prof. R.S. Goudar, Dept. of Botany, R.L. Science Institute, Belgaum. A specimen voucher of the same is deposited in the college museum. The leaves were shade dried at room temperature for 10 days and coarsely powdered with the help of a hand-grinding mill and the powder was passed through sieve No. 60.

### *Preparation of the Extract*

The powder of leaves of *C. esculenta* was extracted separately by continuous hot extraction process using soxhlet apparatus with different solvents in increasing order of polarity from petroleum ether, chloroform, acetone, alcohol, to finally chloroform: water (5). After extraction, the extracts were concentrated under reduced pressure in tared vessel. The marc of crude drug powder was then once again subjected to successive extraction with other solvents and the extractive values were calculated with reference to the air-dried drug. The dried extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

### *Animals:*

Wistar rats of either sex and of approximately the same age, weighing about 150-175 g were used for the study. They were housed in polypropylene cages and fed with standard diet and water *ad libitum*. The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each test, the animals were fasted for at least 12 h. The experimental protocols were subjected to the scrutinization of the Institutional Animal Ethics Committee and were cleared by the same.

### *Acute Toxicity Studies:*

The animals were divided into control and test groups containing six animals each. The control group received the vehicle (1% acacia) while the test groups received graded doses of ethanolic extracts orally (p.o.) and were observed for mortality till 48 h and the LD<sub>50</sub> (6) was calculated.

### *Carrageenan Induced Rat Paw Edema:*

Anti-inflammatory activity was assessed by the method described by Winter *et al* (7). The rats were

divided into three groups of six animals each. First group (negative control) received 1 ml of normal saline; second group (positive control) received 10 mg/kg p.o. indomethacin and third group received ethanolic extract (100 mg/kg, p.o.) of *C. esculenta*, respectively. After 1 h, the rats were challenged with subcutaneous injection of 0.1 ml of 1 % w/v solution of carrageenan (Sigma chemical co, St. Louis MO, USA) into the plantar side of the left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to the mark. The plethysmograph apparatus used for the measurement of rat paw volume was that of Singh and Ghosh (8). The paw volume was measured immediately after injection (0 h) and then every hour till 3 h after injection of carrageenan to each group. The difference between the initial and subsequent reading gave the actual edema volume. Percent inhibition of inflammation was calculated using the formula, % inhibition = 100 (1-Vt/Vc), where 'Vc' represents edema volume in control and 'Vt' edema volume in group treated with test extracts.

### *Carrageenan Induced Pleurisy In Rats:*

The animals were divided into three groups of six rats each as described in the carrageenan induced paw edema model (9, 10) and each were pretreated with ethanolic extract of *C. esculenta* (100 mg/kg, p.o.), Indomethacin (10 mg/kg, p.o.) or normal saline (0.1 ml). One hour later all the animals received 0.25 ml of an intrapleural injection of 1 % carrageenan on the right side of the thorax. The animals were sacrificed 3 h after carrageenan injection by ether inhalation. One ml of heparinized Hank's solution was injected into the pleural cavity and gently massaged to mix its contents. The fluid was aspirated out of the cavity and the exudates were collected. The number of migrating leukocytes in the exudates was determined with Neubauer chamber. The values of each experimental group were expressed as mean  $\pm$  SEM and compared with the control group.

### *Cotton Pellet Granuloma Model:*

In cotton pellet granuloma model the animals were divided into three groups as described in the carrageenan induced paw edema model. The method of Penn *et al* (11) with slight modification was used. The animals were anaesthetized with pentobarbitone (30 mg/kg. s.c.). The back skin was shaved and disinfected with 70% ethanol. An incision was made

in the lumbar region. Subcutaneous tunnels were formed by a blunted forceps and a sterilized, pre weighed cotton pellet was placed on both sides in the scapular region. The animals were treated with indomethacin (10 mg/kg, p.o.) and ethanolic extract of *C. esculenta* for 7 days. Then, the pellets were dissected out and dried until the weight remained constant. The net dry weights, i.e. after subtracting the weight of the cotton pellet were determined.

#### Statistical Analysis:

All values were expressed as mean  $\pm$  SEM. The data were statistically analyzed using one way ANOVA followed by Newman Keul's multiple

range test and differences below  $p < 0.05$  are considered as significant.

### Results

The average percentage yield of the alcohol extract of *C. esculenta* was found to be 2.2 % w/w. Preliminary phytochemical screening of the leaves of *C. esculenta* revealed the presence of alkaloids, flavanoids, saponins and tannins. The LD<sub>50</sub> was found to be 1149 mg/kg for ethanolic extract of *C. esculenta*. So the 1/10 of LD<sub>50</sub> dose was considered as an effective dose.

**Table 1:** Effect of ethanolic extract of *C. esculenta* on carrageenan induced rat paw edema.

Treatment	Dose (mg/kg, p.o.)	Mean increase in paw volume (ml)	% Decrease in paw volume after 3 hrs.
Control (Normal saline)	1 ml	0.37 $\pm$ 0.001	--
Indomethacin	10 mg	0.13 $\pm$ 0.001*	64.86
Extract of <i>C. esculenta</i>	100 mg	0.16 $\pm$ 0.001*	56.76

\*  $p < 0.05$  when compared with control. Values are expressed as mean  $\pm$  SEM (n=6).

**Table 2:** Effect of Ethanolic Extract of *C. esculenta* on Carrageenan Induced Pleurisy in Rats.

Treatment	Dose (mg/kg, p.o.)	Pleural exudates	Leukocytes ( $\times 10^3$ cells/ ml)
Control (Normal saline)	1 ml	0.35 $\pm$ 0.002	4.53 $\pm$ 0.33
Indomethacin	10 mg	0.12 $\pm$ 0.001*	64.86 $\pm$ 0.08*
Extract of <i>C. esculenta</i>	100 mg	0.17 $\pm$ 0.001*	56.76 $\pm$ 0.08*

\*  $p < 0.05$  when compared with control. Values are expressed as mean  $\pm$  SEM (n=6)

**Table 3:** Effect of Ethanolic Extract of *C. esculenta* on Cotton Pellet Induced Granuloma in Rats.

Treatment	Dose (mg/kg, p.o.)	Granuloma wt.	% Inhibition
Control (Normal saline)	1 ml	58.2 $\pm$ 2.04	--
Indomethacin	10 mg	20.7 $\pm$ 0.65*	64.4
Extract of <i>C. esculenta</i>	100 mg	27.3 $\pm$ 0.82*	53.1

\*  $p < 0.05$  when compared with control. Values are expressed as mean  $\pm$  SEM (n=6)

The effect of ethanolic extract of *C. esculenta* on carrageenan-induced edema in rats is shown in Table 1. The results obtained indicate that the ethanolic extract had significant anti-inflammatory activity in rats. The ethanolic extract of *C. esculenta* reduced the edema induced by carrageenan by 56.76 % on oral administration of 100 mg/kg, as compared to the untreated control group. Indomethacin at 10 mg/kg inhibited the edema volume by 64.86 %.

The effect of ethanolic extract of *C. esculenta* on carrageenan-induced pleurisy in rats is shown in Table 2. The volume of pleural exudates in the control group was  $0.35 \pm 0.002$  ml. Animals treated with the ethanolic extract of *C. esculenta* (100 mg/kg, p.o.) decreased the pleural exudates to  $0.17 \pm 0.001$  ml and treatment with Indomethacin (10 mg/kg, p.o.) produced the exudates of  $0.12 \pm 0.001$  ml. The leukocyte count for the control group was found to be  $4.53 \pm 0.33 \times 10^3$  cells/ml. Animals treated with the test extract and standard produced a leukocyte migration of  $0.51 \pm 0.08 \times 10^3$  and  $0.43 \pm 0.08 \times 10^3$  cells/ml, respectively.

The effect of ethanolic extract of *C. esculenta* on cotton pellet induced granuloma in rats is shown in Table 3. In this the mean weights of the cotton pellets were determined. The weight of the granuloma for the control group of animals was found to be  $58.2 \pm 2.04$  mg. Treatment with the ethanolic extract of *C. esculenta* (100 mg/kg, p.o.) decreased the granuloma weight to  $27.3 \pm 0.82$  mg. Treatment with Indomethacin (10 mg/kg, p.o.) produced a granuloma weight of  $20.7 \pm 0.65$  mg. The ethanolic extract of *C. esculenta* and Indomethacin, both inhibited the granuloma tissue formation. The inhibition of the test extract and standard drug was found to be 53.1 and 64.4 %, respectively.

### Discussion

Due to the increasing frequency of intake of NSAID's and their reported common side effects, there is a need to focus on the scientific exploration of herbal drugs having fewer side effects. So, there is a continuous search for indigenous drugs, which can provide relief to inflammation. To give a scientific validation to this plant, an attempt was made to study the anti-inflammatory activity.

Carrageenan induced inflammation is a biphasic phenomenon (12). The first phase of edema is attributed to release of histamine and 5-hydroxy-

tryptamine. Plateau phase is maintained by kinin like substances and second accelerating phase of swelling is attributed to prostaglandin like substances. The knowledge of these mediators involved in different phases is important for interpreting mode of drug action.

The tests performed with the ethanolic extract of *C. esculenta* in the pleurisy model showed that the extract behaves as an inhibitor of leukocyte migration and the formation of pleural exudates when given orally, as reported earlier (13).

In the cotton pellet granuloma model, inflammation and granuloma develops during the period of several days. This model is an indication for the proliferative phase of inflammation. Inflammation involves proliferation of macrophages, neutrophils and fibroblasts, which are basic sources of granuloma formation. Hence, the decrease in the weight of granuloma indicates that the proliferative phase was effectively suppressed by the ethanol extract of *C. esculenta*.

Thus it can be concluded that the leaves of the plant *C. esculenta* possess significant anti-inflammatory activity in rats. Further studies involving the purification of the chemical constituents of the plant and the investigations in the biochemical pathways may result in the development of a potent anti-inflammatory agent with low toxicity and better therapeutic index.

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